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**CHRONOAMPEROMETRIC DETECTION OF GLUCOSE BY A THIRD GENERATION
BIOSENSOR CONSTRUCTED FROM CONDUCTING MICROTUBULES OF
POLYPYRROLE**

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ABSTRACT

Chronoamperometry is used to characterize a previously described amperometric glucose sensor based on conducting microtubules of poly(pyrrole). With the chronoamperometric technique glucose concentrations in the range of 1-15 mM can be measured accurately. The procedure may lead to a prolonged sensor lifetime.

INTRODUCTION

The employment of conducting polymers in amperometric biosensors has been studied extensively over the past few years [1-3]. The simultaneous exploitation of the electronic properties and the enzyme immobilizing capacities of conducting polymers has received little attention so far. Immobilization of enzymes on conducting polymers has been reported, but the results have been disappointing because the enzymes lose their activity [4,5].

In a previous paper we reported that conducting microtubules of poly(pyrrole) can adsorb glucose oxidase very efficiently. We showed that the immobilized enzyme is able to communicate directly with the conducting matrix. We have constructed a so-called third-generation glucose sensor from these microtubules [6]. This sensor detects glucose amperometrically without the need of a co-substrate or a low molecular weight mediator as in first [7] and second [8] generation biosensors. The lifetime of the sensors was found to be very long. In a subsequent paper we presented evidence that the microporous environment and the morphology of the interior of the poly(pyrrole) microtubules are important for realizing the direct electronic communication between the enzyme and the conducting polymer [9].

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In this paper we describe the chronoamperometric detection of glucose by our biosensor. This detection method involves the recording of a current as a function of time, just after a transient in potential has been applied. During the actual measurement the potential is kept constant. We will show that chronoamperometric measurements can be utilized successfully to measure various concentrations of glucose without heavily burdening the sensor. This is important for increasing the lifetime of the sensor device. In Figure 1 a schematic representation of our biosensor is given. Also is shown how the electrons involved in the enzymatic oxidation of glucose are transferred to the conducting polymer and eventually to the anode where they are measured as a current.

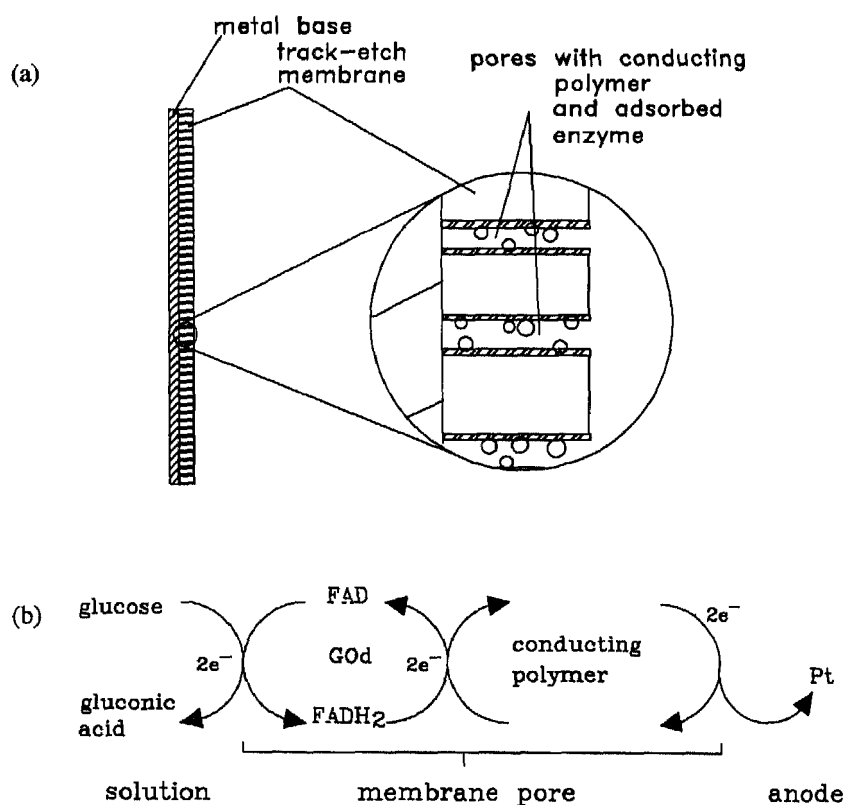


Figure 1. (a) Schematic representation of the track-etch biosensor; (b) electron shuttle, showing the path of the electrons involved in the enzymatic oxidation of glucose, mediated by the poly(pyrrole) microtubules.

EXPERIMENTAL PROCEDURES

Details regarding the synthesis of microtubular poly(pyrrole) and the construction of the biosensor have been described elsewhere [9,10]. Electrochemical measurements were carried out with an Antec CU-04-AZ electrochemical controller (Antec Leyden, The Netherlands). The current output was recorded on a Kipp model BD 111/112 pen recorder (Kipp & Zonen, The Netherlands).

For the chronoamperometric measurements the enzyme membrane was placed as the working electrode in a home-made 3-electrode cell. An Ag/AgCl wire was used as a quasi-reference electrode and a platinum wire was used as the auxiliary electrode. The cell volume was 1 ml. Phosphate buffered saline (PBS) solution (pH 7.4) was used as the electrolyte in the electrochemical measurements. Before the chronoamperometric measurements were conducted, the current output of the biosensor was allowed to stabilize at a potential of 350 mV vs. Ag/AgCl quasi-reference. During the measurements the cell was switched off for a fixed period of time. In this switch-off time the open cell voltage (in mV vs Ag/AgCl) was recorded with a Handykit MK-601 digital multimeter. After switching on at a potential of 350 mV vs. Ag/AgCl the resulting current response was recorded for 5 min. Different glucose concentrations ranging from 0 to 15 mM were used in the experiments.

RESULTS AND DISCUSSION

The amperometric glucose sensor was constructed as described in our previous papers [6,9,11]. The sensor membrane was placed as the working electrode in a 3-electrode cell. Before use the biosensor was poised at a potential of 350 mV vs. Ag/AgCl during 12 hrs. to stabilize the output current to a minimum value. After this period the current had dropped to approximately 100 nA. The chronoamperometric measurements were performed by switching the cell off for a certain period of time and then switching it on again at a potential of 350 mV vs. Ag/AgCl. Subsequently, the resulting current response was recorded for 5 minutes. Various concentrations of glucose in phosphate buffered saline solution were measured. The 'switch-off' time was varied from 1 to 30 minutes. At the end of the 'switch-off' period the open cell voltage of the cell was measured. This was done for the following reason. When a conducting polymer electrode is poised at a certain potential, large currents will flow in the beginning because of capacitive charging of the polymer. These charges become localized inside the polymer matrix [12-14]. As a result, a so-called memory effect occurs [15]. The open cell voltage does not correspond to the equilibrium potential, i.e. the potential which is measured directly after incorporating the sensor into the cell, but is dependent on the potential it had been poised at before. The result of this effect can be seen in Figure 2, which shows the open cell voltage as measured at the end of various 'switch-off' periods. At short 'switch-off'

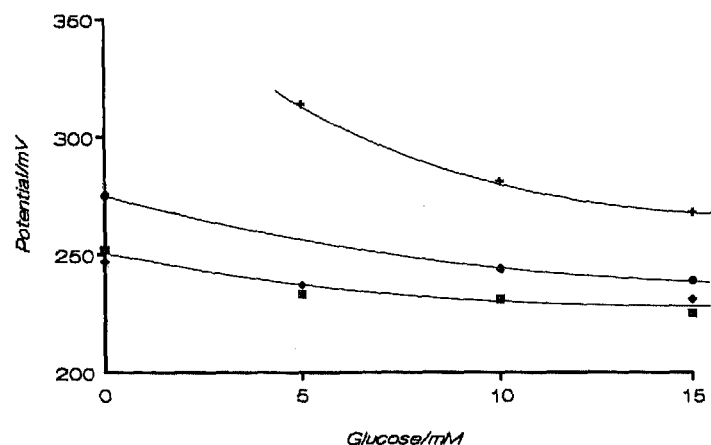


Figure 2. Open cell voltage of the biosensor at the end of various 'switch-off' times as a function of the glucose concentration in the cell: (+) 1 min., (●) 3 min., (◆) 10 min., (■) 30 min.

times the memory-effect is clearly present, whereas at longer times the potential has diminished to more or less the equilibrium potential (225 mV vs. Ag/AgCl).

The current resulting from switching the biosensor on after a switch-off time of 30 minutes at a potential of 350 mV vs. Ag/AgCl is shown in Figure 3. As can be seen different decay curves result when different glucose concentrations are measured. After approximately 5

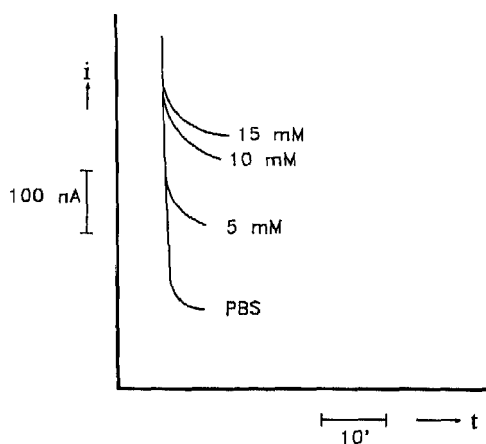


Figure 3. Response current of the biosensor as a result of a potential step from the open cell voltage (225 mV) to 350 mV as a function of time at various glucose concentrations. The cell had been switched off for 30 min. Potentials versus Ag/AgCl reference.

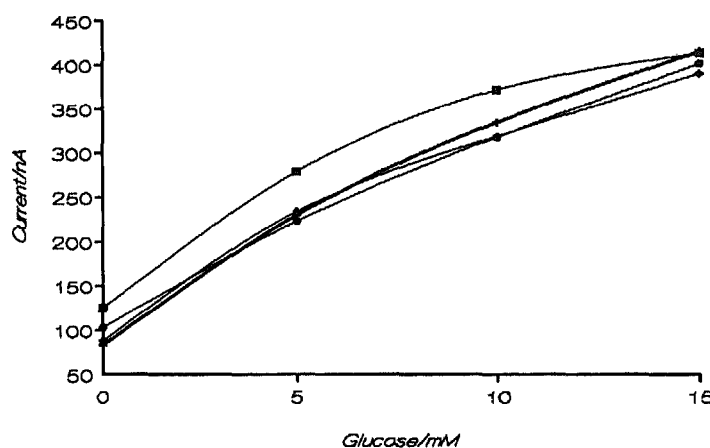


Figure 4. Plot of the chronoamperometric steady-state current at 350 mV (vs. Ag/AgCl) as a function of glucose concentration after various 'switch-off' times: (+) 1 min., (●) 3 min., (♦) 10 min., (■) 30 min.

minutes a steady-state current is reached, which can be used to determine the glucose concentration in the cell. With this method we were able to measure concentrations of glucose up to 15 mM. Higher concentrations gave curves that could not be distinguished from the curve for 15 mM glucose. In Figure 4 the steady-state current values, obtained after switch-off times ranging from 1 to 30 min., are plotted as a function of the glucose concentration. The curves are rather similar. Therefore, we can conclude that it is possible to switch the biosensor off for a relatively long time and still obtain a fast and accurate reading of the glucose concentration. This discontinuous measurement procedure may be advantageous for two reasons. First, the biosensor functions by oxidizing the glucose enzymatically. This means that glucose molecules are consumed, leading to a depletion of substrate at the sensor surface. In a continuous measurement substrate must be transported from the bulk solution to the biosensor surface in order to obtain a reliable sensor output. With chronoamperometry, the measurement is discontinuous and diffusion controlled. As a result, it is possible to obtain a reliable response even in stagnant solutions. Second, applying a potential to the electrode for a long period of time may degrade the conducting polymer [16-18]. Although the electrode potential used in our biosensor is low and will not have a large effect on the short run, it may be deleterious on the long term. The discontinuous character of the chronoamperometric measurement may, therefore, enhance the lifetime of the biosensor.

CONCLUSIONS

In this paper we have shown that it is possible to determine glucose concentrations in the range of 1 - 15 mM by means of chronoamperometry, using a third-generation biosensor constructed from microtubular poly(pyrrole). The measurements can be carried out successfully by this technique, despite the occurrence of large capacitive current contributions. The sensor can be switched off between measurements for a long period of time. This is advantageous, as it may increase the life-time of the device.

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